Exploring factors in honey bee choice of antimicrobial plant resins

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Background

• Honey bees (*Apis mellifera*) are social insects that live in large managed colonies, for pollination and honey production purposes. Honey bees are known to collect plant resins for various purposes (Simone et al 2009).

•When hive is challenged with pathogens, such as *Ascosphaera apis,* the fungus responsible for chalkbrood disease, the hive will collect more propolis.

• Honey bees in US mostly collect resin from the leaf buds of Eastern Cottonwood (*Populus deltoides*), although other resin producing plants, such as White Spruce (*Picea glauca*), have been shown to have higher antimicrobial levels against chalkbrood (Wilson et al 2013).

• Resin choice may be determined by many factors: bioavailability due to cottonwood buds surface area, or volatiles emitted by resins that bees are able to smell.

Question: do bees have a preference for cottonwood over spruce resin?

To test this question, we tried to determine if honey bees were able to learn the odor of cottonwood resin more quickly than that of spruce resin.

Null Hypothesis: No difference in learning curves to bees presented with cottonwood or spruce odor.

Methods: Cage experiments

• Small bee hives (nucleus colonies) were placed inside an outdoor mesh cage to prevent outside resin foraging.

• Resin extracts prepared by placing cottonwood leaf buds and spruce bark samples in jars filled with 100% ethanol. Jars were placed on shaker for one week for full dissolution of resins in ethanol. Other plant materials were filtered out of the resin extract after one week.

• Cottonwood and spruce resins, along with a control of no resin, were placed equal distances away from the entrance of the hive above sugar feeders, allowing the odor of the resin to be associated with sucrose solution reward.

• Bee visitors to each resin were marked and recorded. Arrangement of resin feeders was changed periodically throughout trials.

• Experiments repeated with new bee colony.

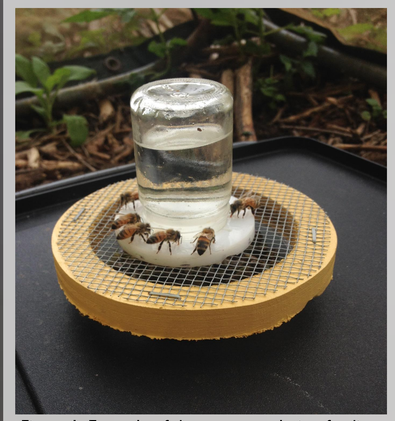

Figure 1: Example of the sucrose solution feeding apparatus. The wire mesh allowed for resin volatiles to escape, leading bees to a sugar reward.

Figure 2: Outdoor mesh cage with nucleus colony inside. One sugar feeder is visible behind the colony.

Methods: proboscis extension response (PER)

• Bees collected from Bee Lab colony, chilled on ice, and harnessed using straws and duct tape. 39 bees were tested for each odor.

• Harnessed bees incubated at 26° C, 50% relative humidity for an hour before trials.

• Bees placed into carousel; ambient air was allowed to blow on bees for thirty seconds before odor presented.

• Odor presented in association with sugar reward.

• Two seconds after odor delivery, 1molar sucrose solution was touched to the bee's antennae to elicit proboscis extension.

• Each bee was presented with the odor of cottonwood or spruce resin a minimum of 7 times.

• Learning was recorded if the bee extended its proboscis upon presentation of the odor before antennae was touched with sugar.

Figure 3: Harnessed bee receiving sugar reward after extending proboscis in response to resin odor.

Results

• The cage experiment's Colony I showed a significant preference to cottonwood odor over spruce in the first three trials. Colony II responded more weakly overall.

•A chi-squared test used to compare the first three trials of the Colony I experiments gave a p-value of 0.008.

• In PER trials, the peak learning response occurred for both spruce and cottonwood at the 5th presentation of the odor. The percentage of bees displaying the learned response was higher for cottonwood resin.

•5 of the 39 bees tested with cottonwood did not display any learning result; in spruce tests, 12 of the 39 bees had no learning response.

•A Mann-Whitney *U* test was used to compare the overall sum of proboscis extension to both resins; the test gave a p-value of 0.060.

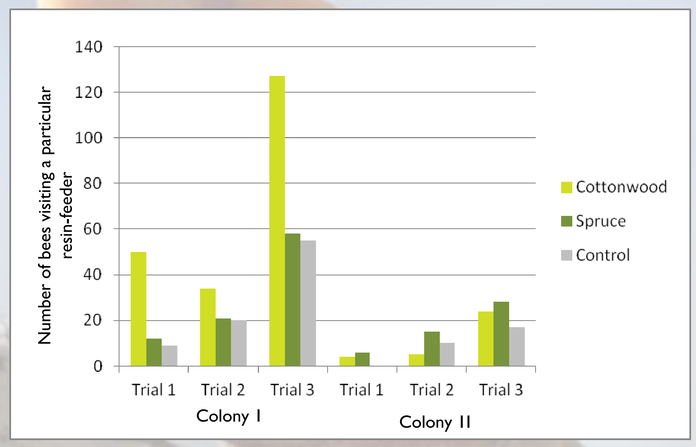

Figure 4: Comparison of bee visitation to different resins and their attached sugar feeders. The highest number of bees visiting a particular resin-feeder (the cottonwood feeder from Trial 3 of Colony 1) was 127.

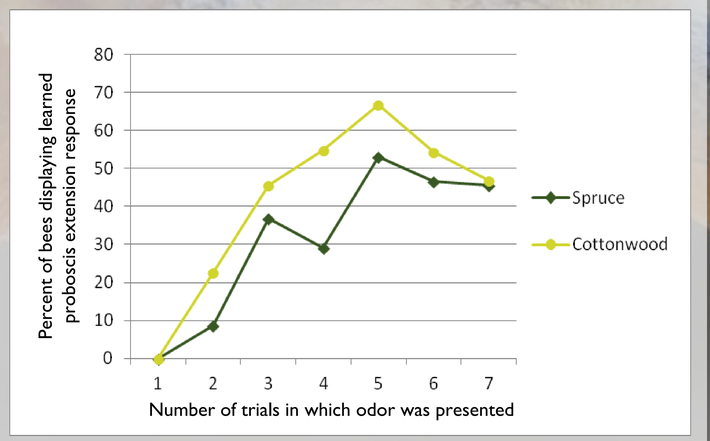

Figure 5: Learning curves in response to continued presentation of resin odor in association with sugar reward. The max percentages of bees displaying learned response for cottonwood resin and spruce resin, respectively, were 72.2% and 90%, respectively. Rate of learning peaked in cottonwood at trial 5 and decreased significantly in subsequent trials.

Discussion

• Cage trial response varied between bee colonies; the first colony responded more strongly to both resins and the control than the second colony.

•Seasonal availability of resins and changing need to use propolis potential factors in cage trial variation.

• Honey bees in PER trials learned cottonwood odor consistently faster than spruce odor.

• Though the Mann-Whitney comparison of PER response to each resin gave a p-value too large to be considered statistically significant, faster learning of cottonwood over spruce is still a noticeable trend. Future studies could benefit from similar trials with a larger overall sample size.

• In addition to bees' ability to detect resin volatiles, availability of resins from both cottonwood and spruce may play a large role in the amount of resins bees collect from each species.

Works Cited

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Wilson MB, Spivak M, Hegeman AD, Rendahl A, Cohen JD. 2013. Metabolomics reveals the origins of antimicrobial resins collected by honey bees. PLoSOne 8(10): e77512. doi: 10.1371/journal.pone.0077512

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